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A standardised protocol for sampling cavity-nesting Hymenoptera using trap nests

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Abstract

Hymenoptera are among the most important members of temperate ecosystems, both for their contribution to ecosystem services and their role in overall ecosystem functioning. While they have received considerable research attention due to their ecological significance and recent declines, substantial knowledge gaps remain regarding their ecology, phenology, and population trajectories.

Trap nests are a popular sampling tool for cavity-nesting Hymenoptera, offering excellent potential to fill these knowledge gaps by simultaneously providing data on species-abundance and key ecological, phenological, and behavioural processes. Trap nests also serve as an excellent tool for monitoring environmental change, habitat restoration efforts, and other interventions. However, their use has not yet been standardised, limiting data sharing and comparability between studies.

Here, we introduce the first step-by-step protocol for producing and implementing trap nests, available in its entirety via protocols.io. With this protocol and the provided supplementary resources, we aim to promote the use of trap nests and facilitate the standardisation of cavity-nesting Hymenoptera data collection, which is essential for advancing research and developing conservation measures effectively.

Keywords

Brood cell, brood parasites, guide, kleptoparasites, natural enemies, solitary wild bees and wasps, stem-nesting Hymenoptera

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Introduction

Due to their crucial roles in ecosystem functioning and recent population declines, particularly in agricultural areas, Hymenoptera have become the subject of considerable research attention (Biesmeijer et al. 2006; Klein et al. 2007; Ollerton et al. 2011; IPBES et al. 2016; Sánchez-Bayo and Wychkuys 2021; Quandahor et al. 2024). Cavity-nesting Hymenoptera (solitary bees and wasps) are of particular conservation concern due to their crucial roles in providing ecosystem services, such as pollination and pest control (Veddeler 2006; Kleijn et al. 2015; Bosch et al. 2021; Brock et al. 2021). However, our knowledge regarding the life history, ecology, and population trajectories of many species remains incomplete, hindering effective conservation efforts (Marshall et al. 2024).

Trap nests are uniquely suited to fill these knowledge gaps, as they allow for the collection of diverse ecological and species-level data. (Staab et al. 2018). They consist of pre-drilled holes or hollow tubes that mimic the natural nesting environment of cavity-nesting bee and wasp species, enticing females to establish nests that can later be retrieved and inspected (Krombein 1967; see Fig. 1). These nests provide data on provisioned food sources, nest materials, and natural enemies of the nesting species, thereby shedding light on key behavioural, ecological, and phenological processes (Albrecht et al. 2007; Sabino and Antonini 2017; Ivanov et al. 2019). They have been used in behavioural and natural history studies to reveal species' foraging and reproductive behaviour (Gathmann and Tscharntke 2002; Ganser et al. 2020; Fornoff et al. 2023; Crone et al. 2024; Timm et al. 2024). In recent decades, ecological studies have made increasing use of trap nests to study trophic interactions (such as predation, and parasitism) and to assess interaction diversity within communities (Tscharntke et al. 2003; Albrecht et al. 2007; Staab et al. 2018; Mayr et al. 2020). Trap nests can also function as "living laboratories", offering opportunities to study ecological dynamics in real-time, including the monitoring of emerging diseases and pathogen transmission (Kierat et al. 2017). The diverse array of data provided by trap nests fundamentally differentiates them from other widely used sampling methods for flying and flower visiting insects – such as transect sampling, Malaise-traps and pan traps – which capture adult individuals and thus rarely provide more than simple species abundance data (Westphal et al. 2008). Trap nests also offer the advantage of generating almost a year's worth of data with minimal field effort during the peak field season, requiring only two field days – one for deployment and one for collection. This makes trap nests particularly well suited for difficult-to-access habitats such as wetlands and areas with dense vegetation, where frequent site visits are impractical.

By sampling nests rather than individual adults, trap nests ensure that only resident populations are sampled, unlike other methods that also capture transient individuals (Gathmann and Tscharntke 2002). Therefore, trap nests provide a more representative assessment of local community composition, making them highly effective tools for bioindication (Tscharntke et al. 2003; Grixti and Packer 2006; MacIvor 2017; Staab et al. 2018; Klaus et al. 2023). Trap nests are frequently used in agricultural environments to track the impact of intensification or ecological restoration efforts on

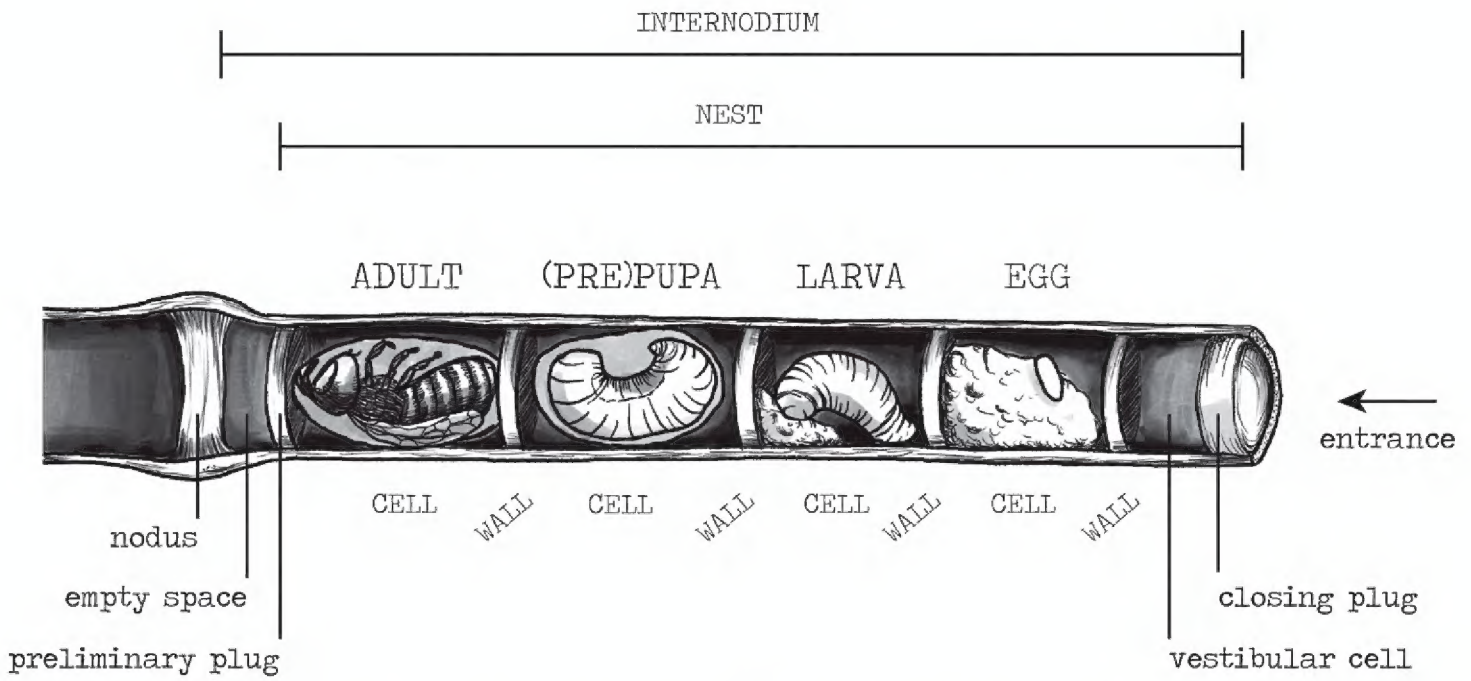

Figure 1. A schematic representation of a nest and the four main stages of development: egg, larva, prepupae or pupae, and imago (adult). Nests contain one or more brood cells, each housing one offspring along with food provisioned by the mother: nectar and pollen in bee nests and paralysed arthropods (spiders, insects and their larvae) in wasp nests. In most species, brood cells are separated by partition walls and the nest opening is sealed off by a closing plug. Species will overwinter as pre-pupae or as fully developed adults in cocoons (in the case of bees) or pupal cuticles (in the case of wasps).

cavity-nesting Hymenoptera, as these species provide critical ecosystem services such as pollination and biological pest control (Albrecht et al. 2007; Fabian et al. 2013; Kovács-Hostyánszki et al. 2021; Nilsson et al. 2021). Although these data are invaluable, the utility of trap nests as bioindicators is limited by the narrow range of species they sample (Westphal et al. 2008).

While some excellent practical resources on trap nest use exist (e.g., Fricke 1991; MacIvor 2017; Staab et al. 2018; Lindermann et al. 2023), there are no publicly available step-by-step protocols, despite their growing popularity (Staab et al. 2018). This lack of standardisation hinders data sharing and comparisons across datasets, ultimately slowing both research progress and the development of effective conservation measures (Levenson et al. 2024; Marshall et al. 2024). Here, we introduce a detailed step-by-step protocol and identification guide that we developed for the use of trap nests. The protocol is available in its entirety via protocols.io.

Methods

This protocol and the accompanying identification guide were developed through the collaboration of two research groups and a university department, drawing on field experience from several monitoring programs in Hungary and Romania between 2016 and 2023 (e.g., Báldi et al. 2022). Expert input and user feedback gathered over several years were incorporated to further refine the protocol. Efforts were made to ensure its

accessibility to research groups working across a wide range of temperate ecosystems. Below, we provide a brief overview of the trap nest production and sampling method and the associated data curation process.

In our protocol, we construct trap nests using reed stems that are bound together and placed in a tube casing. Reed trap nests are widely used because they attract a wide range of species and provide easy access to nests, while still being cost effective and easy to produce (MacIvor 2017; von Königslöw et al. 2019). Cutting reeds between internodes produces clean cavities – typically two per stem – that can be individually colonized, and their natural variation in inner diameter (recommended range from 2 to 12 mm) accommodates a broad range of species (MacIvor 2017; von Königslöw et al. 2019; Gilpin et al. 2022). The optimal length of reed pieces will depend on the internode spacing of the available reeds; however, cutting length should be chosen to maximise the yield of reed pieces containing exactly one node while ensuring sufficient cavity length so as not to negatively impact colonisation or skew sex ratios (Alonso et al. 2012; Seidelmann et al. 2016; MacIvor 2017; Ferreira et al. 2025).

When deployed in early spring, trap nests are attached to trees or poles, and a protective sheet is placed above them to shield against weather damage. We recommend placing trap nests in pairs to ensure coverage in case one is damaged, and to allow one to remain in the field, thereby reducing the risk of removing the majority of the local cavity-nesting hymenopteran communities. The correct placement of trap nests and timing of their retrieval are crucial for ensuring unbiased sampling (see the full protocol and studies cited within for further guidance). In autumn, nests are retrieved from the field and stored at room temperature until winter, when they are transferred to cold storage for overwintering, allowing offspring to fully develop while limiting further damage by natural enemies.

From mid-winter, we start processing the trap nests. First, all unoccupied reeds are removed, followed by a more thorough inspection of occupied reeds, during which data entry and sample collection take place. Nests should be processed before offspring emerge from their nests, as certain data are best collected while nests are intact. However, processing nests early means that some species will still be in the larval or pre-pupal stage, which is less ideal for species identification. Therefore, once the nests have been inspected and data have been recorded, all reeds containing larvae should be closed back up and stored at room temperature for further development, allowing for the collection of adult samples and for more accurate species identification. While molecular species identification can be valuable in supplementing traditional identification methods, exclusive reliance on it is likely not feasible due to high costs (Fornoff et al. 2023). The almost two-year process of producing and obtaining data from trap nests is visualised in Fig. 2.

Results

Building on years of field and laboratory experience, we developed a step-by-step protocol describing our trap nest methodology, which is available via protocols.io. It describes trap nest production and use (i.e., the deployment, retrieval, and storage of trap nests), followed by reed sorting and data entry. Finally, the process of putting away samples and the develop-

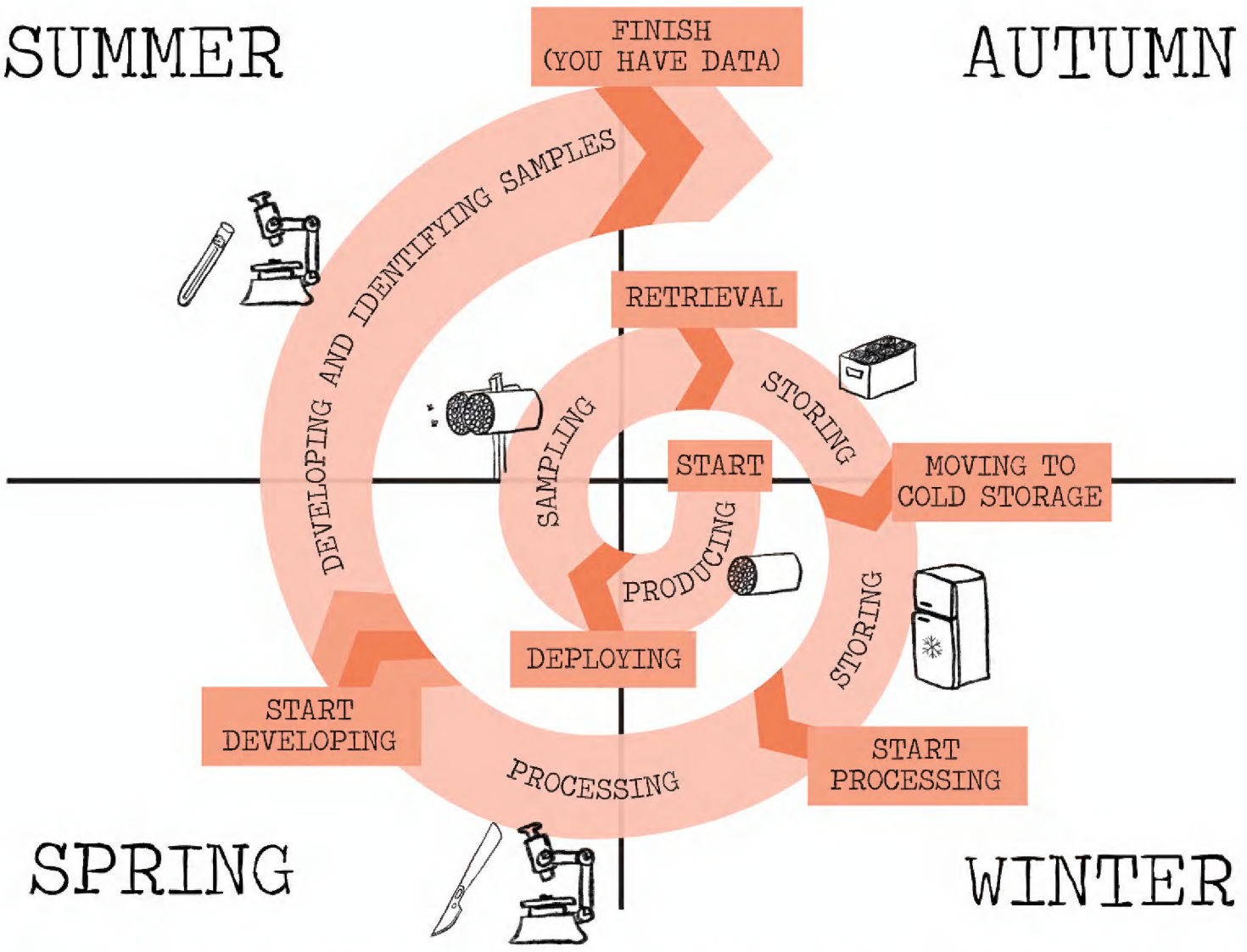

Figure 2. Schematic representation of the nearly two-year process from producing trap nests to obtaining data.

ment of immature broods is described. Comments and user feedback can be submitted on protocols.io and will be used to improve future versions of the protocol. The protocol link contains three attachments: two spreadsheets that provide templates for data entry during nest processing and an identification guide that provides help with the identification of common East-Central European cavity-nesting taxa and their natural enemies. Of the two datasheets, the Status datasheet is for trap-nest-level administrative tracking, and the Processing datasheet is for nest-level data such as taxa identity and nest properties (provisioned food type, nest material, etc.). The identification guide provides detailed descriptions of nest properties and other taxon-specific characteristics of larval or adult specimens, derived from our own empirical observations. These features often provide crucial clues for species-, genus-, or family-level identification – even in the absence of fully developed offspring.

Discussion

The protocol and identification guide we have provided do not require any prior knowledge of trap nests or species identification, which facilitates the involvement of non-experts, such as lab assistants, students and early-career researchers, and reduces the need for supervision. Previous studies have demonstrated that

non-experts can be trained to collect trap nest data and identify taxa with a high degree of accuracy (Rondeau et al. 2023; Lindermann et al. 2024). As such, the protocol can be readily adopted by research institutions, conservation authorities, and citizen science projects alike, broadening its applicability in both academic and applied settings.

The use of standardised protocols such as ours will facilitate data sharing and collaboration between research institutes. However, inherent sampling biases towards certain species associated with trap nests may still be present. For example, insects that nest earlier in the season will likely have more nesting opportunities (empty reeds) available to them. Conversely, nests of these species are also exposed to adverse conditions and are available to potential natural enemies for longer, which may cause higher mortality in early-nesting species. Future research should investigate if such biases exist and whether they can be avoided, for example, by replacing occupied reeds throughout the sampling period. Additionally, distinguishing brood cell status can be challenging; for example, an empty cell may have been parasitised or may not have contained offspring to begin with. This subjectivity is a potential source of interobserver variability and will need to be addressed through further research into the phenology and nesting biology of many trap nest species. Although the timing of steps in our protocol is based on the seasonal development of temperate cavity-nesting Hymenoptera, it can be adapted for use in tropical regions, provided there is appropriate knowledge of the local species' phenology.

While this protocol is tailored to the use of reeds as nesting substrate, the fundamental steps are not exclusive to this type of trap nest. In particular, bamboo can be equally effective as reeds when suitable inner diameters are ensured (Barthélémy 2012; Yoon et al. 2015; von Königslöw et al. 2019). We recommend reeds or bamboo because they avoid the drawbacks of other common methods. For example, wooden blocks with pre-drilled holes are highly attractive to females of cavity-nesting species but these nests cannot be easily extracted (MacIvor 2017). Conversely, plastic and paper tubes allow easy access to nests but plastic tubes are associated with high brood mortality (Fye 1965; Stephen and Every 1970), while paper or cardboard tubes are prone to being damaged by weather or wildlife (MacIvor 2017). Pithy plant stems, such as those of the genera *Sambucus* (e.g., elderberry) and *Rubus* (e.g., blackberry), have also been used; however, they are associated with high mortality and low occupancy rates (von Königslöw et al. 2019). Finally, nesting boxes composed of stacked wooden boards with grooves are a popular design that allows for non-lethal sampling (Hallett 2001; Lindermann et al. 2024); although, they may be susceptible to parasite infestations due to gaps between the boards (Eeraerts et al. 2022).

Our protocol offers a standardised and efficient method for using trap nests. Its flexibility and ease of use make it accessible to non-professionals and applicable across a broad range of research contexts. Continued refinement through user feedback via protocols.io and the investigation of possible sampling biases will further enhance its value, contributing to the development of a globally harmonised framework for monitoring cavity-nesting Hymenoptera.

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